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Differential Diagnosis of Schistosomiasis: Future Approaches, Limitations and Challenges

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ABSTRACT
Background: Schistosomiasis, recognized as the second most detrimental parasitic disease in tropical regions, has considerable public health implications in areas where it is endemic. Numerous disease control strategies, especially mass drug administration (MDA) implemented through school-based or community-wide initiatives, have an important impact in decreasing infection transmission rates among high-risk populations. However, one of the primary barriers to successful disease control is the absence of reliable diagnostic tools. It is widely acknowledged that current diagnostic practices for schistosomiasis largely depend on traditional techniques. Despite this, minor disease symptoms, alongside the limited sensitivity and specificity of these conventional diagnostic methods, continue to present significant challenges for healthcare professionals. Consequently, there is a pressing need to develop innovative, practical, and more accurate diagnostic tools and biomarkers to enhance the detection of this infection. Obtaining affordable, user-friendly, and precise diagnostic solutions for schistosomiasis remains a significant hurdle, limiting the scope of initiatives for comprehensive disease control. Along with striving to create novel diagnostic methods, efforts should also focus on refining the existing tools to improve their effectiveness.
Objective: This review explores current diagnostic approaches for schistosomiasis, emphasizing their limitations and challenges while suggesting potential future methods. Additionally, it aims to examine the biomedical tools used for identifying and diagnosing biomarkers related to schistosomiasis.
Conclusions: The success of efforts to eliminate schistosomiasis is heavily reliant on the precise detection of infections using highly sensitive diagnostic tests, particularly in cases of low-intensity infections. Adequate sanitation infrastructure is lacking, especially in tropical and subtropical regions, highlighting the urgent need for biomedical tools can identify and diagnose new biomarkers for schistosomiasis.

Keywords: schistosomiasis; diagnostic challenges; diagnostic tools; diagnostic limitations.

1. INTRODUCTION

Schistosomiasis is a chronic parasitic disease caused by trematode worms, commonly (blood flukes), which belong to the genus Schistosoma. The infection is transmitted to humans, who serve as the definitive hosts, through contact with fresh water contaminated by intermediate snail hosts. It ranks as one of the most significant parasitic diseases impacting public health and the economy in tropical and subtropical regions, following malaria in importance. Schistosomiasis predominantly affects marginalized populations, particularly those lacking access to clean water and adequate sanitation. According to the World Health Organization (WHO), an estimated 236.6 million people required preventive treatment for schistosomiasis in 2019, with over 105.4 million receiving such treatment. Notably, at least 90% of individuals needing treatment are located in Africa, where schistosomiasis is responsible for an estimated 300,000 deaths annually **[1-3].**

The geographical overlap between schistosomiasis and soil-transmitted helminthiases (STHs) often leads to co-infections or independent occurrences, depending on the interaction of environmental risks and host-specific factors. Risk factors include limited availability of clean water and poor hygiene practices of caregivers and children, which increase vulnerability to STH infections. School-aged children (SAC) are at higher risk of contracting schistosomiasis as a result of engaging in unsafe water-related activities. In contrast, preschool-aged children (PSAC) are typically exposed when accompanying their parents during daily water collection or chores **[4- 6].** The transmission cycle of schistosomiasis begins when infected human excreta containing parasite eggs contaminates freshwater sources. In such environments, the eggs hatch into miracidia, which infect snails. Within these snails, the parasites develop into sporocysts and later produce cercariae. Humans become infected when cercariae penetrate the skin during contact with contaminated water **[1, 7].**

Diagnosis of schistosomiasis primarily involves the identification of parasite eggs in urine or stool samples. Blood and urine samples may also be tested for the presence of antibodies or antigens as markers of infection. For urogenital schistosomiasis, the most common method involves filtering urine samples through materials such as nylon, paper, or polycarbonate filters. In cases of Schistosoma haematobium infection, microscopic traces of blood in urine are almost always present and can be detected using chemical reagent strips. Diagnosing intestinal schistosomiasis typically requires stool samples analyzed via the Kato-Katz method, which uses methylene blue-stained cellophane or glass slides. In areas endemic toS. mansoni, the circulating cathodic antigen (CCA) test can also be employed. For populations in non-endemic or low-transmission areas, serological and immunological tests may assist in detecting past exposure, determining the need for further examination, and guiding treatment and follow-up measures**[2, 5, 8].**This review explores the diagnostic methods for detecting schistosomiasis, identifying its limitations and challenges, and highlighting advancements and future directions in the field. The text also examines different biomedical tools for diagnosing and identifying schistosomalbiomarkers.

2. Epidemiology, Differential Diagnosis, and Pathogenesis of Schistosomiasis in a Perspective Glance.

Schistosomiasis, called bilharzia, is a neglected tropical disease caused by blood-dwelling parasitic worms called trematodes. This disease primarily affects populations in low-income countries within tropical and subtropical climates. Approximately 250 million people contract schistosomiasis each year. It is widespread across tropical regions worldwide and holds the rank of the second most significant human parasitic disease in terms of socio-economic consequences, coming after malaria. The WHO reports that schistosomiasis is endemic in 78 countries, where regular preventive drug treatment is needed in 51 due to moderate to severe transmission levels**[9, 10].** The infection is prevalent in regions such as Africa, South America, and Asia, where access to safe drinking water and improved sanitation is often unavailable. High-risk groups include rural populations engaged in water-based agriculture, particularly women and children who depend on contaminated water for household tasks such as cooking and washing**[2, 11].**

2.1. Epidemiology of Schistosomiasis

Human schistosomiasis arises from five primary pathogenic species: Schistosoma mansoni, Schistosoma japonicum, Schistosoma intercalatum, Schistosoma mekongi, and Schistosoma haematobium. Among these, S. mansoni, S. japonicum, and S. haematobium are the most frequently observed worldwide. The transmission of S. mansoni depends on freshwater snails from the genus Biomphalaria as intermediate hosts, while its reservoirs include certain mammals, such as primates, marsupials, and rodents. Adult S. mansoni parasites reside in the lower mesenteric veins, producing between 200 and 300 eggs daily. Eggs not excreted with feces often become trapped in host tissues, triggering granulomatous inflammation. The distribution of S. mansoni spans Africa, the Middle East, the Caribbean, South America—including Brazil and Venezuela—and other tropical regions**[1, 12].**

Schistosoma japonicum has a particularly high reservoir diversity, infecting animals such as cattle, dogs, cats, rodents, pigs, horses, and goats. Its intermediate host is the freshwater snail of the genus Oncomelania. In humans, adult S. japonicum worms reside in the upper mesenteric veins and deposit eggs capable of reaching the liver or intestines. This species is associated with a more rapid onset of liver fibrosis and, occasionally, liver failure compared to other hepatotropic schistosomes. Its geographic presence includes China, the Philippines, and Sulawesi in Indonesia **[6,13].**

Schistosoma haematobium is unique in causing urogenital disorders in humans. This parasite is primarily found in Africa and the Middle East, with primates acting as its main reservoir hosts. Adult females deposit eggs in pelvic blood vessels, causing pathology in vital urogenital organs. Notably, the International Agency for Research on Cancer (IARC) has classified this species as a likely human carcinogen. In contrast, S. intercalatum and S. mekongi exhibit more regionally restricted distributions. S. intercalatum is predominantly found in the Democratic Republic of Congo, sharing the same intermediate snail host (Bulinus) as S. haematobium.Meanwhile, S.mekongi is restricted to areas in Cambodia and Laos, infecting snails from the genus Neotricula **[2, 14, 15].**

2.2. Schistosomiasis Life cycle

Schistosomes are dioecious parasitic worms that inhabit the bloodstream and follow a complex life cycle involving both intermediate and definitive hosts. Understanding this cycle is vital for devising effective control measures against schistosomiasis **[16,17].** The main stages of the schistosome life cycle are as follows**Figure 1:**

- **Egg Stage:** Adult female worms deposit eggs in the blood vessels of their definitive host. Depending on the species, daily egg production varies significantly; for instance, S. mansoni produces 100–300 eggs per day, while S. japonicum can produce 500–3,500 eggs.

- **Miracidium Stage:** These eggs exit the host's body through feces or urine. Upon contact with freshwater, the eggs hatch into free-swimming larvae called miracidia, which must find and infect a specific freshwater snail species to continue developing.
- **Snail Intermediate Host:** Inside the snail, miracidia undergo asexual reproduction, transforming into cercariae. For example, Biomphalaria snails host S. mansoni, while Oncomelania snails are S. japonicumhosts.
- **Cercariae Stage:** Cercariae are released back into the water and can infect humans by penetrating the skin during water contact.
- **Schistosomula Stage:** Once inside the human host, cercariae lose their tails and transform into schistosomula. These develop further as they migrate through the bloodstream to the lungs and then to the liver.
- **Adult Stage:** In the liver, schistosomula mature into adult worms. The paired male and female worms migrate to the preferred blood vessel environments, such as the mesenteric veins for S. mansoni or the pelvic veins for S. haematobium.
- **Cycle Repeats:** Adult worms produce eggs that are excreted in urine or feces, continuing the cycle**.**

Schistosomes possess a unique survival adaptation evading them from the host immune system. They absorb and mimic the host's antigens on their surfaces, an example of molecular mimicry that enables them to persist for several years inside their host. Depending on their species, these parasites can locate themselves in the intestinal or bladder plexus.About 1 to 3 months after cercarial penetration, eggs begin to be produced by female worms. While some eggs are excreted, others remain trapped in host tissues, provoking granulomatous inflammatory responses**[18, 19].**

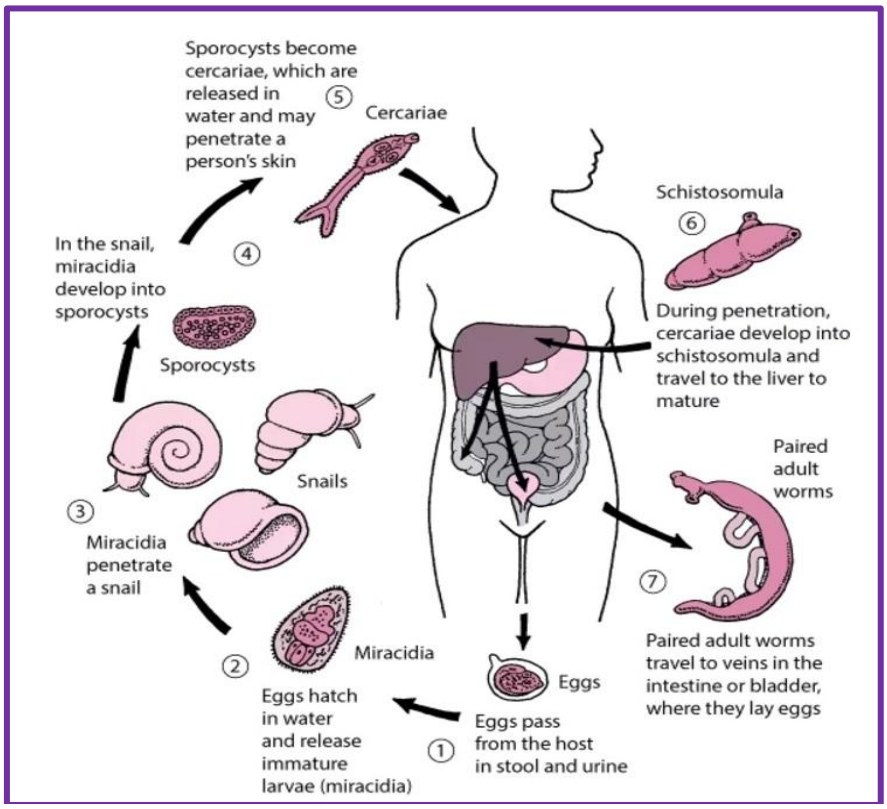

Figure 1: Schistosoma life cycle**(20).**

2.3. Diagnosis of Schistosomiasis

The diagnosis of schistosomiasis depends on specific epidemiological factors and symptoms associated with the acute or chronic stages of the disease**[21].** For acute schistosomiasis, healthcare providers should evaluate recently returned travelers from endemic regions who present with symptoms such as nighttime fevers, a dry cough, headaches, fatigue, rash, digestive distress, and organ enlargement (e.g., hepatosplenomegaly). These symptoms overlap with acute viral, bacterial, or malarial infections.Some distinguishable features are generalized hives, an itching rash at the site of cercarial penetration (commonly on the legs), and elevated eosinophil levels**[22,23].**

Neurological symptoms, including focal deficits, may occur in some acute cases, necessitating the consideration of differential diagnoses such as neuro-schistosomiasis, neurocysticercosis, or coccidioidomycosis.

Differentiation is achieved using patient history, imaging studies, and cerebrospinal fluid analyses. Patients from endemic regions presenting with eosinophilia and gastrointestinal or urogenital symptoms like hematuria, painful urination, blood in semen, or painful intercourse may have chronic schistosomiasis resulting from exposure to contaminated freshwater. Co-existing parasitic helminth infections, such as clonorchiasis, fascioliasis, trichinosis, paragonimiasis, strongyloidiasis, hookworm, or ascariasis, are also common in endemic areas and may contribute to overlapping symptoms like fever and eosinophilia**[17].** Urinary schistosomiasis caused by S. haematobium leads to hematuria, which must be distinguished from urinary tract infections, acute nephritis, renal tuberculosis, or cancers of the urogenital system. Similarly, alternate explanations should be considered when evaluating infertility in cases of suspected genital schistosomiasis. International guidelines recommend diagnostic screenings for HIV antibodies, HCV antibodies, HBs antigen, HAV antibodies, Strongyloides antibodies, and latent tuberculosis in individuals diagnosed with bilharzia**[2,24].**
Laboratory indicators supporting schistosomiasis include eosinophilia, IgE assay, calprotectin, fecal occult blood, and proteinuria. Diagnosis can be based on direct or indirect testing. Direct methods identify parasites via egg detection in stool, urine, or tissue biopsies, and antigenic and molecular tests targeting schistosomal DNA. Indirect tests, such as antibody detection in serum, also aid diagnosis. Parasitological tests are commonly performed using microscopy to detect Schistosoma eggs in biological samples of tissues **Figure 1**like intestine or bladder**[21].**

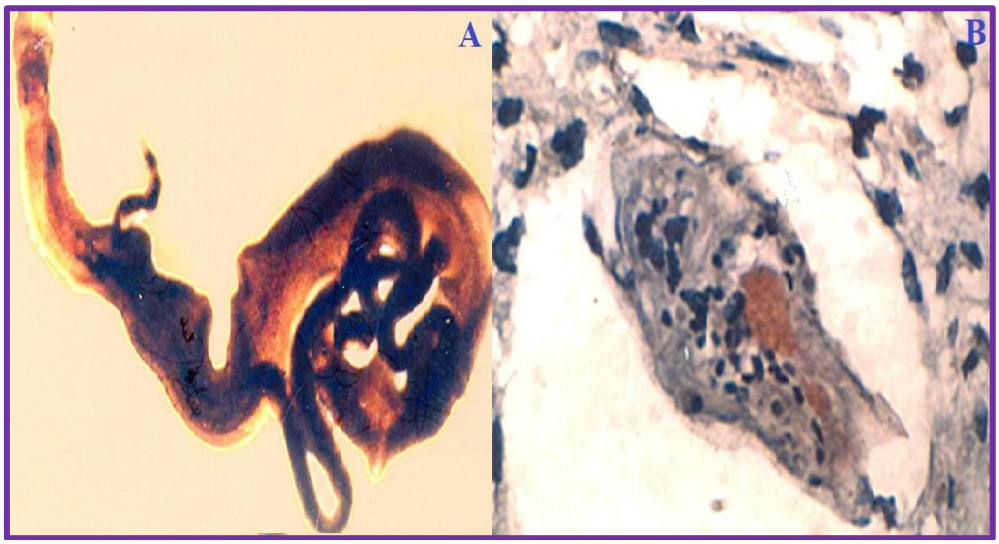

Figure 2: Schistosoma adult worms couple (A), and granuloma's reaction in host tissue (B), **(our work materials).**

2.4. Pathogenesis and Clinical Manifestations of Schistosomiasis
Schistosomiasis presents in acute or chronic forms, with the acute stage including cercarial dermatitis and Katayama syndrome. The latter, named after the Katayama District in Japan where S. japonicum was first documented in 1904, is triggered by systemic hypersensitivity to antigens from the schistosomes or their eggs**[17,25, 26].** Cercarial dermatitis, characterized by a maculopapular rash at the point of larval penetration, typically appears within 2 to 7 days of exposure. This condition is self-limiting and resolves within two weeks, due to the host's hypersensitive response.Katayama syndrome manifests with symptoms such as fever, abdominal pain, diarrhea, myalgia, chest tightness, cough, and pulmonary infiltrates, often accompanied by eosinophilia. It is predominantly noted in travelers or inhabitants of highly endemic areas. Symptom onset occurs weeks to months post-exposure and improves within 2–10 weeks, although severe cases may lead to persistent effects like weight loss and hepatomegaly [10, 25].
Repeated exposure in endemic regions typically causes chronic schistosomiasis as acute episodes are uncommon. The chronic phase involves adult parasites producing eggs that lack surface antigens, making them vulnerable to inflammatory responses. Host CD4+ and Th-2 lymphocytes respond to egg-derived glycoproteins, contributing to granulomatous inflammation Figure 12and fibrotic lesions, especially in liver and bladder tissues. While praziquantel therapy can resolve active infections, chronic disease often leads to diminished immune responses to egg antigens[27,28].

2.5. Intestinal and Urogenital Schistosomiasis
Due to its persistent nature, chronic schistosomiasis poses significant public health challenges. The disease's severity correlates with egg burden in tissues. Intestinal schistosomiasis caused by S. mansoni, S. japonicum, S.

intercalatum, and S. mekongi can begin with bloody diarrhea and anemia linked to mucosal ulceration. Advanced cases may progress to portal hypertension, polyposis, hepatic fibrosis, or cirrhosis **[16, 23,29].**
Urogenital schistosomiasis, caused by S. haematobium, is associated with calcified eggs in the bladder and genital structures. This leads to polyposis, hyperplasia, ulcerations, and potential hemorrhaging. Advanced stages may result in bladder calcification, urethral obstruction, hydronephrosis, and renal failure. Chronic bladder inflammation induced by egg granulomas is linked to transitional tissue damage and bacterial infections, which generate nitrosamines capable of promoting carcinogenesis. Due to this, S. haematobium is regarded as a class 1 carcinogen by the IARC for its association with bladder squamous cell carcinoma (SCC)[23,30].
Additionally, chronic inflammation associated with female genital schistosomiasis (FGS) has been implicated in infertility and increased risk of HIV transmission. This form of schistosomiasis causes severe ulceration and fibrosis in reproductive organs. Fragile, inflamed epithelium makes it easier for HIV to spread. Several studies suggest that praziquantel treatment, by controlling schistosomiasis, may lower HIV susceptibility and viral load [23, 31]. Schistosomiasis is a condition with potentially severe outcomes ranging from gastrointestinal and urogenital complications to increased risks of infertility and cancer. Proper diagnosis relies on a thorough understanding of epidemiological factors, clinical presentations, and laboratory testing. By addressing both acute and chronic forms, healthcare systems can mitigate the global burden of this parasitic disease, especially in endemic areas[6,32].

3. Schistosomiasis Diagnosis

Schistosomiasis is a disease that can be both prevented and treated. Effective diagnostics play a pivotal role in enabling early intervention, saving countless lives, especially among small-scale agricultural and fishing communities. Significant global efforts are underway to eliminate schistosomiasis as a major public health issue, particularly in combating neglected tropical diseases. Updated guidelines by the WHO in 2022 emphasize an integrated approach to control and eventually eliminate human schistosomiasis [1, 2, 6]. This recommended strategy includes extending preventive treatment to all at-risk individuals aged two years and older in communities with a prevalence rate of 10% or higher. The approach also incorporates sanitation measures, treatment at health facilities, and snail control to curb the disease. Sub-Saharan African countries, where most at-risk populations reside, have scaled up treatment campaigns. Assessments of mass drug administration initiatives in 2022 demonstrated a noticeable decline in schistosomiasis prevalence in countries such as Burundi, Eritrea, Eswatini, Gambia, Lesotho, and Rwanda [33].
To bolster diagnostic capabilities for neglected tropical diseases like schistosomiasis, WHO set up a diagnostics technical advisory group (DTAG) in October 2021. This group aims to create target product profiles (TPPs) that specify the requirements for innovative diagnostic tools. For schistosomiasis, WHO guidelines prioritize diagnostics tailored to S. mansoni or S. haematobium, with a preference for tools capable of detecting both species. The ideal diagnostic test should be deployable at the point-of-care (POC), requiring minimal infrastructure, and should facilitate decisions during a single field visit. WHO has emphasized that the cost of the POC test should not exceed $3 per sample, making it significantly more economical than laboratory-based tools, which may require startup capital of up to $10,000 [5,8].Schistosomiasis diagnosis in endemic regions primarily relies on microscopy-based techniques to detect schistosome eggs. These methods involve stool and urine sample analysis prepared using the Kato-Katz (KK) and urine filtration techniques, enabling the identification of intestinal and urogenital schistosomiasis[34].

3.1. Diagnostic Tests for Human Schistosomiasis

Detection of Circulating Proteins: Adult schistosomes and their eggs release specific proteins that circulate in bodily fluids such as blood, urine, stool, and saliva. Diagnostic tests have been developed targeting circulating anodic antigen (CAA) and circulating cathodic antigen (CCA). Both antigens are excreted through the patient's urine and indicate active infections**[23,35].** Additionally, antibody production resulting from schistosome infections can act as a diagnostic marker. The detection of immunoglobulin G (IgG) and immunoglobulin M (IgM), produced in response to cercarial secretions, egg antigens, or adult antigens, is commonly utilized in antibody-based diagnostic methods. These methods are particularly sensitive and useful for individuals with low-intensity infections but face challenges such as false positives. Persistent antibody circulation after cleared infections and cross-reactivity with antigens shared by other helminth species like Filaria spp., Echinococcus spp., and Strongyloides remain key limitations**[25,36].**
Nucleic Acid-Based Diagnostics: Nucleic Acid Amplification Tests (NAATs), which amplify specific genetic regions of schistosomes, represent another advanced diagnostic tool. Commonly employed NAATs include conventional PCR, real-time PCR, and LAMP (loop-mediated isothermal amplification assay). Methods such as digital droplet PCR, nested PCR, and recombinase polymerase amplification (RPA) have also been developed. These assays offer significantly greater sensitivity than microscopy, making them suitable diagnostic tools in low-prevalence settings **[5, 8,37].**

3.2. Biomarkers Detection in Schistosomiasis Diagnosis

Antigen and Antibody-Based Biomarkers: Antigen-based biomarkers are among the most widely studied for schistosomiasis diagnosis. CAA and CCA, glycoconjugates associated with the gut of adult worms, have been extensively explored in this domain. To address limitations in antigen characterization, particularly for CCA, researchers have shifted toward developing monoclonal antibodies (MAbs). These MAbs have enabled more precise identification of antigen epitopes, broadening the scope of diagnostic techniques **[35,38].** The soluble egg antigen (SEA) is another critical biomarker. SEA, released by eggs deposited in the liver, modulates immune responses by promoting the Th2 pathway and suppressing the Th1 pathway. Antibodies targeting schistosomiasis antigens, such as the major egg antigen (MEA), have been identified through proteomic analyses as promising biomarkers for detecting infections **[39, 40].**

Other Protein-Associated Biomarkers: Schistosomal eggs elicit primary inflammatory responses as they adhere to mesenteric vessels, and intestinal tissues, or are deposited in the liver. The associated immune response involves granuloma formation and inflammation, leading to portal hypertension and fibrosis. Cell adhesion molecules (CAMs), which include selectins, cadherins, integrins, and ICAM-1, play a crucial role in facilitating inflammatory and immune responses during schistosomal infections. Selectins and cadherins, in particular, play key roles in leukocyte adhesion to inflammation sites and granuloma development **[41, 42].**

3.3. Key Challenges in Schistosomiasis Diagnosis

Genus-Specific POC Diagnostics: Despite the availability of a single-drug treatment (Praziquantel) for schistosomiasis, the development of genus-specific POC diagnostic tests remains critical. Though modern PCR assays have exhibited promise, their utility in remote settings is limited by the need for expensive equipment, DNA extraction protocols, and cold chains. Efforts to redefine POC diagnostics have focused on CCA and CAA as biomarkers. The UCP-LF-CAA test has demonstrated enhanced sensitivity compared to microscopy and qPCR, with its dry reagent format allowing easy storage and transport **[23, 38,43].**

Sensitivity and Specificity Issues: Schistosomal diagnosis with conventional microscopy (limited sensitivity) has increased the demand for more reliable methods in low-prevalence settings. Although advanced tests like NAATs and POC-CCA tools outperform microscopy, challenges such as false negatives can hinder accurate prevalence data and erode elimination strategies **[44, 45].**

Challenges in Diagnosing Genital Schistosomiasis: Misdiagnoses regarding male and female genital schistosomiasis (FGS) remain prevalent. According to WHO, FGS disproportionately affects women, with an estimated 56 million women and girls in the African region potentially impacted. Tailored diagnostic efforts and critical awareness are essential to address unique clinical signs and complications of FGS **[5, 46].**

Nature of Samples Used for Schistosomiasis Diagnosis: To enhance diagnostic sensitivity, the World Health Organization (WHO) advises using urine samples for urogenital schistosomiasis and stool samples for intestinal schistosomiasis. Both sample types are non-invasive for patients; however, their odor can be off-putting for healthcare workers, potentially limiting the frequency of disease monitoring. Exploring alternative samples—such as blood serum, dried blood spots (DBS), finger-prick blood, and saliva—poses challenges due to concerns about invasiveness and reduced sensitivity, depending on the diagnostic test employed **[8,47].**

Diagnostic Target Product Profiles (TPPs) and Reassured Criteria for Schistosomiasis: The WHO noted in 2021 that many national health agencies lack effective tools to successfully confirm schistosomal control programs. Current gold-standard diagnostic approaches often require trained personnel, microscopes, and stool samples, which can be inconvenient for healthcare practitioners. To address these constraints, WHO developed Target Product Profiles (TPPs) to facilitate the creation of improved diagnostic tests. These TPPs aim to enhance the specificity and sensitivity of diagnostic methods while enabling the use of non-stool or non-venous blood samples, such as saliva, urine, or finger-stick blood **[5, 8].**

3.4. Accuracy of Diagnostic Tests for Schistosomiasis

The WHO continues to employ standard tools such as the Kato-Katz stool test and urine filtration for identifying schistosome eggs in stool and urine samples. While these approaches remain widely used for diagnosing intestinal and urinary schistosomiasis, they exhibit low sensitivity for detecting low-intensity infections. Addressing this limitation, serological techniques have emerged for identifying antibodies against schistosome antigens, including methods like indirect immunofluorescent-antibody tests (IFATs), indirect hemagglutination assays (IHAs), and enzyme-linked immunosorbent assays (ELISAs) **[7,48].**

Additionally, molecular diagnostics such as PCR, multiplex PCR, qPCR, and LAMP are increasingly favored for their precision and sensitivity in detecting parasite DNA. Detecting cell-free DNA (cfDNA) in host blood also holds promise as a diagnostic marker for schistosomiasis. To identify optimal diagnostic tools, particularly for low-prevalence settings, further examination of diagnostic performance for S. mansoni and S. haematobium is essential. For S. haematobium, comparative studies have demonstrated higher diagnostic accuracy with PCR (97% sensitivity, 94% specificity) than with urine CCA (sensitivity of 53% and specificity of 81%). Limited

data on SmCTF antibody tests precludes meta-analysis, but sensitivity values from two studies were reported as 67% and 100%, with specificities of 39% and 45% **[23, 44, 45].**

3.5. Advances in Schistosomiasis Diagnosis

Diagnostic approaches for schistosomiasis now extend beyond conventional methods to include highly sensitive techniques. The standard diagnostic protocol integrates medical history, physical exams, lab tests, and radiology. Clinical morbidity markers, including subclinical and biochemical indicators, offer indirect diagnostic evidence, though they often lack specificity **[41].** Laboratory methods are categorized into direct and indirect techniques. Direct parasitological techniques identify parasite ova in feces, urine, or tissue, while direct immunological tests detect schistosome antigens in bodily fluids. Conversely, indirect immunological approaches detect parasite-specific antibodies in blood samples**[22,42].**

Conventional Parasitological Diagnosis: Traditional diagnostic strategies focus on examining stool and urine via microscopy to detect parasite eggs. Methods like the Kato-Katz technique and urine sedimentation remain the gold standard for quantifying infection intensity, particularly in endemic regions. While these approaches are cost-effective and require minimal training, they have critical drawbacks, including low sensitivity for light-intensity infections and labor-intensive workflows. Advances such as formol-ether sedimentation, salt flotation, and novel techniques involving magnetic microspheres continue to refine fecal analyses.However, these approaches, though inexpensive, are time-consuming and less effective in detecting light infections **[5, 8,41,49].**

Malacological Surveys: The assessment of snail intermediate hosts for schistosomiasis is still underutilized, even though it is quite important. Malacological surveys generally involve detecting sporocysts and cercariae through snail dissection or isolating snails in controlled environments to examine cercarial shedding. While these methods effectively uncover mature infections, they often underestimate true prevalence in areas with low transmission rates or mixed infections**[50- 52].**

Immunological Diagnosis: Immunological assays supplement conventional diagnostics by detecting infections that might otherwise result in false negatives, particularly in low-intensity cases. These tests are useful in research and preliminary screenings in endemic areas, where traditional methods fall short following mass drug administration programs. While indirect immunodiagnostic assays are highly sensitive and effective for disease surveillance, antibodies targeted in such tests can persist long after the infection has resolved, possibly leading to diagnostic inaccuracies**[22, 23, 41].**

ELISA stands out as a principal serological tool with remarkable sensitivity and specificity in diagnosing schistosomiasis. This technique involves binding soluble antigens or antibodies to the surface of multi-well plates, enabling the detection of various antibody classes using diverse antigens. Through antigen-antibody affinity, ELISA delivers qualitative or quantitative results. Initially, Schistosoma antigen detection employed crude soluble egg antigens (SEA) and soluble adult worms' proteins (SWAP). Over time, this approach evolved to use more refined excretory/secretory antigens. Detecting SEA and SWAP in serum or excreta provides valuable diagnostic potential due to their correlation with parasitic load, facilitating early treatment initiatives **[40, 44, 45].** Compared to other diagnostic tools, immunological assays for antigens or antibodies are simpler and particularly useful for identifying early infections with high specificity. Additionally, antigens derived from schistosomula or cercariae have proven effective for early infection detection, despite challenges in antigen extraction from these sources **[7].**

3.6. Limitations of Current Diagnostic Techniques for Schistosomiasis

Despite advancements, existing diagnostic methods for schistosomiasis face significant limitations. The integration of new tools into routine field and facility-based settings remains challenging due to cost and adaptability concerns, particularly in low-income regions where healthcare access is limited **[49].**Surveillance and accurate diagnosis are critical for controlling schistosomiasis, yet traditional direct parasitological methods, while considered the gold standard, are labor-intensive, time-consuming, and ill-suited for large-scale surveillance. These methods, which rely heavily on egg excretion rates, demonstrate reduced sensitivity in low-prevalence areas, often resulting in false-negative outcomes. This limitation constrains early detection and treatment benefits offered by drugs like praziquantel. To enhance diagnostic accuracy, repeated testing during follow-up evaluations is often recommended, as no single test guarantees precise results **[7,41,45].**

The Kato-Katz technique remains the benchmark diagnostic tool for schistosomiasis due to its simplicity and cost-efficiency, making it a preferred choice for epidemiological studies and surveillance as endorsed by the WHO. However, its limitations include inadequate detection of low-intensity and early-stage infections as well as restrictions in assessing therapeutic outcomes **[44].** Immunological methods, despite demonstrating

comparable sensitivity and specificity to ELISA, cannot distinguish between active and past infections, potentially leading to unnecessary treatments. Immunodiagnostic assays are instrumental in detecting antibodies against schistosomal antigens but are often challenged by the persistence of antibodies post-treatment, which cannot indicate successful eradication of the infection**[53, 54].**

Molecular diagnostic approaches, such as detecting schistosome DNA in fecal, blood, or urinesamples, offer high sensitivity and are more effective in identifying infections with low parasite loads. However, these methods are expensive, require advanced resources, and are constrained by sample preparation and processing limitations. PCR-based methods exhibit increased positivity rates compared to Kato-Katz or miracidium hatching but are unable to analyze larger sample volumes, limiting their clinical utility. Additionally, molecular diagnostics depend heavily on expensive infrastructure, specialized training, and cold chain logistics, making their field application—in resource-limited settings—impractical **[41, 55, 56].**

3.7. Future Approaches in the Diagnosis of Schistosomiasis

Efforts to combat schistosomiasis are evolving, with a push toward eliminating transmission through improved diagnostic methods. Significant emphasis is placed on developing diagnostic tools that are cost-effective, highly sensitive, and specific, particularly for low-endemic settings. While current diagnostic tools provide critical support for control and elimination programs, challenges persist, including financial constraints and limited commercial incentives for test development in endemic, resource-constrained regions**[42,44,54].**

The costs associated with diagnostic tests, including equipment, reagents, labor, and logisticalsupport, pose significant challenges, especially for low-income countries. Additionally, there is limited private sector investment in diagnostic tools targeting neglected tropical diseases like schistosomiasis, further hindering advancements in this area. Affordably achieving high diagnostic sensitivity remains an enduring challenge for stakeholders and health authorities in endemic regions, requiring international collaboration to address financial and logistical barriers**[22,41,53].**

Though molecular diagnostic methods like PCR and qPCR outperform conventional and serological approaches in sensitivity, their adoption is stymied by high costs, lengthy processing times, and infrastructure requirements. Future analytical techniques must balance cost-efficiency with practical execution, focusing on affordability without compromising diagnostic accuracy. Furthermore, false-positive and false-negative rates remain problematic, necessitating enhanced test validity and robust differential diagnostics to guide accurate treatment decisions **[10, 56].**

4. Conclusions

Schistosomiasis remains a critical public health issue, predominantly affecting populations in tropical and subtropical areas with inadequate sanitation. The parasite Schistosoma spp. undergoes a complex life cycle, modulating various host biomarkers during infection. Achieving effective control or elimination of schistosomiasis requires reliable diagnostic tools that can sensitively detect infections, particularly in low-transmission settings. The precision of diagnostic methods directly impacts public health interventions and elimination strategies, with a clear need for continuous investment in developing cost-effective, efficient, and accurate diagnostic tests.

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Data Availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Declaration

Ethical considerations

Ethics approval and animal considerations followed internationally agreed ethical best practices in clinical veterinary research (CVR) as outlined in the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The author declares that she has no competing interests.

Author Contributions
Fatemah Enad Alajmi is responsible for all the steps of manuscript preparation.

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